

Characterization of Heat-tolerant Microbial Species Isolated from Spacecraft Assembly Facility

K. Venkateswaran¹, A. Vu¹, M. Musick¹, C. Echeverria¹, S. Chung¹, M. Satomi², R. Koukol¹, R. Kern¹, and D.C. White¹; ¹Jet Propulsion Laboratory, Calif. Inst. Tech., Pasadena, CA 91109; ²Natl. Res. Inst. Fish. Sci., Yokohama, Kanagawa 236, JAPAN

In ongoing investigations to map and archive the microbial footprints in various components of spacecraft and its accessories, we have examined the microbial populations of the Spacecraft Assembly Facility (SAF). We have exposed witness plates that are made up of spacecraft materials and or painted with spacecraft-quality paints for ~7 to 9 months. In the initial studies reported here, we have examined the total cultivable aerobic heterotrophs, and heat-tolerant (80°C for 15-min.) spore-formers. The results showed that the witness plates coated with spacecraft quality paints attracted more dust particles than the non-coated stainless steel witness plates. Among four paints tested, witness plates coated with NS43G accumulated the highest number of particles, hence attracted more cultivable microbes and spore-formers. The microbiological examination revealed that the SAF High Bay-1 harbors mainly Gram-positive microbes and mostly spore-forming *Bacillus* species. Most of the isolated microbes were heat resistant to 80°C for 15 min., grew well at 60°C, and in the presence of 10% NaCl concentration. Some of the isolates were resistant to H₂O₂ treatment that kills majority of the microbes. Based on the morphology, and physiology, 28 isolates were chosen for further study. The phylogenetic relationships among these heat-tolerant microbes were examined using a battery of morphological, physiological, and molecular characterizations. Among 28 strains tested for their phenotypic characterization, only 8 strains were identified as to species. This may be due to the fact that the commercially available identification kit was not designed to identify unknown bacterial species that are found in the extreme environment. The fatty acid methyl ester profiles of 80% of the tested isolates matched with known bacterial species. Sequence analysis of nearly complete sequences of 16S ribosomal RNA revealed that most of the microbes in SAF are *Bacillus licheniformis*. By 16S rRNA analysis, the isolates fell into seven clades *Bacillus licheniformis*, *B. pumilus*, *B. cereus*, *B. circulans*, *Staphylococcus epidermidis*, and representatives of the genera *Planococcus* and *Micrococcus*. In addition to the *Bacillus* species (22 isolates), the isolation of human-associated microbes such as *S. epidermidis* indicates secondary contamination by human activity in SAF. DNA-DNA hybridization studies were carried out between the SAF strains and species procured from various culture collections.

[†] The research described in this paper was carried out by the Jet Propulsion Laboratory, California Institute of Technology, under contract with the National Aeronautics and Space Administration.